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# ESTIMATION AND STABILITY STUDIES UNDER VARIOUS CONDITIONS OF FELODIPINE BY HPLC METHOD

***[1]Dillip Kumar Mohapatra, [1]Swarupamayee Kalta, [1]Deepika Rani Panigrahi[1], Prasanta Kumar Sahoo, [1]Somejeet Panda***

[1]Department of Pharmaceutical Analysis, Gayatri College of Pharmacy, Sambalpur, Odisha

ABSTRACT :

With the recent regulatory requirements ICH Q2 (R2) and ICH Q14, is progressing, and it must be able to manage the entire life cycle of the methodology. This is also applicable to and especially challenging for combinations of drug. The aim of the present work is to develop and validating the stability studies in various conditions and estimation of felodipine using HPLC. The determination was carried out on advanced rapid resolution HPLC instrument. This carried out on kramacil C18 (4.5 mm × 25 cm, 5µm) column using a mobile phase of phosphate buffer water-pH-3-4: acetonitrile (20:80V/V). The flow rate was 1.0ml/min with detection at 273nm. Initially a HPLC method was developed and partially validated with all the parameters such as Linearity, Accuracy, and LLOD&LLOQ, Injection precision & Intraday-Interday variations. Method to be proven to be effective in all of the above parameters. Drug showed perfect linearity in the range of 100-6.25µg/ml with $R^2 = 0.9971$ , method was sensitive even lower concentration such as 200ng/ml and shown RSD value for injection precision 1.8 which was within the suggested limit for bioanalytical compounds. By exposed to direct sun light up to 5 hrs and subjected to HPLC, the result is no degradation occurred. Under acidic condition our result was cleared that felodipine was degraded under (2 N HCL: Acetonitrile) after 5 min. Also stability of drug was evaluated under different thermal condition. From our result it was cleared that felodipine was stable at 55°C up to 2hrs.

**Kew words: -** ICH, HPLC, Linearity, Accuracy, LLOD&LLOQ, felodipine

## Introduction :

Felodipine is under class of Calcium Channel Blocker used in the treatment of myocardial infarction, heart failure [1, 2]. Calcium is necessary for muscle cells to contract. Felodipine prevents calcium from being released within the muscle cells of the small arteries and thereby causes the muscles to relax and the arteries to dilate or expand. Dilation of arteries reduces blood pressure. Felodipine has little or no effect on the muscles of veins or the heart.[3-6]Felodipine chemically is Ethylmethyl(4RS)-4-(2,3-dichlorophenyl)-2,6-dimethyl-1,4dihydropyridine-3,dicarboxylate with molecular formula C18H19Cl2NO4 and molecular weight 284.3.

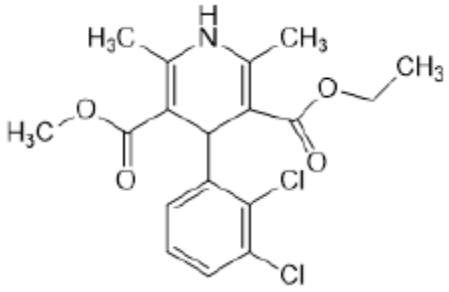



**Fig-1, Chemical structure of felodipine**

Several methods were reported for the determination of FEL individually using UV spectrophotometry [7], HPLC [8], spectrofluorimetric [9], gas chromatography [10], and electrochemical methods [11]. The pharmacological activity depends on both, the integrity of the chemical structure and the stereochemistry, which must be retained to develop the optimal interaction with the receptor and thus get the pharmacological and therapeutic efficacy. Obvi- ously all changes in the pharmacophore induces changes in its pharmacological or toxicological properties [12]. For these reasons the study of drug stability acquires an important pharmaceutical relevance, and is an important factor to determine both efficacy and toxicity. Commonly it is not enough to know the drug amount declared in pharmaceuticals, but is desirable to know if degradation occurred and the kind of the degradation products

[13]. Taking into account that as a consequence of drug degradation generally minimal chemical changes are produced, selective analytical tools for the quantification and/or identification of the degradation products are required. Objective of current studies is to validated of Felodipine in bulk drug to evaluate felodipine stability under various condition.

## 2. Experimental

### *2.1. Reagents and drugs*

Acetonitrile, Ultra pure water and all other reagents employed were of analytical
or HPLC grade.and Felodipine were obtained from Onan Biotech, Hyderabad.Marketed formulation was procured from local market.

### *2.2 .Apparatus*

Felodipine was analyzed using HPLC system consisting of Agilent1200 series RRLC separations module with EZchrom Elite software, auto injector and agilest photodiode array detector (DAD). Separation was carried out using a Waters Symmetry C18 column (25cm × 4.5mm, 5µ,). Isocratic elution was carried with the mobile phase consisting of a mobile phase acetonenitrile and water (pH 3) (80:20) at flow rate of 1ml/min. Mobile phase was filtered (Millipore system, 0.2µm) under vacuum and degassed. Chromatographic separation was monitored at 273 nm. All the samples were analyzed at room temperature. Total run time for the analysis was 10 minutes.

### *2.3.Preparation of stock solutions :-*

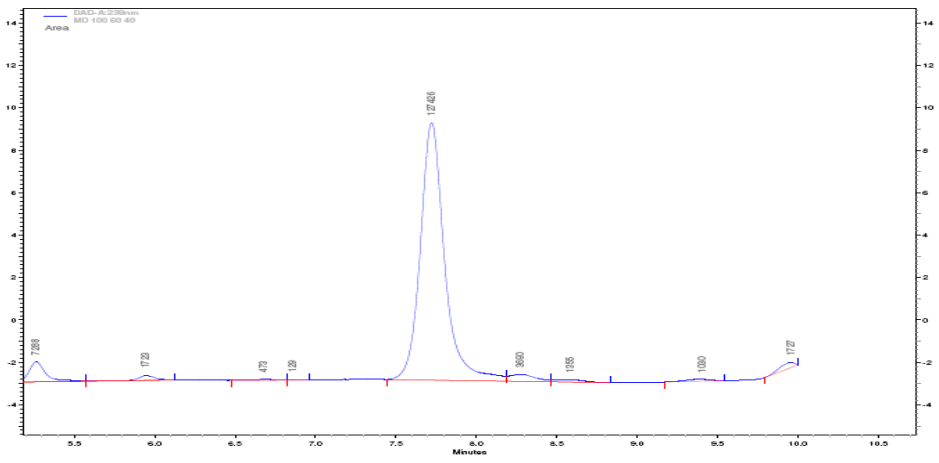
Primary standard stock solutions of Felodipine was prepared by dissolving accurately weighed amount (10mg) of drug in 1ml mobile phase to produce a concentration of 10 mg/ml. working standard solutions of analytes were prepared by appropriate dilution of stock solutions in mobile phase to get 100µg/ml. The further concentrations required for constructing calibration curve were prepared by dilution of 100µg/ml working standard.

## 3. Method development:

Method was trial with taken same concentration of sample and analyze at different ratio of solvent concentration and select a good precise method.

**Table:1**

| Acetonitrile:Water(pH3) | Flow rate(ml/min) | Retention time(RT)(min) | Peak Area |
|---|---|---|---|
| 60:40 | 1.0 | 7.73 | 127426 |
| 70:30 | 1.0 | 5.80 | 6204130 |
| 75:25 | 1.0 | 7.00 | 5733258 |
| 80:20 | 1.0 | 5.47 | 6277374 |



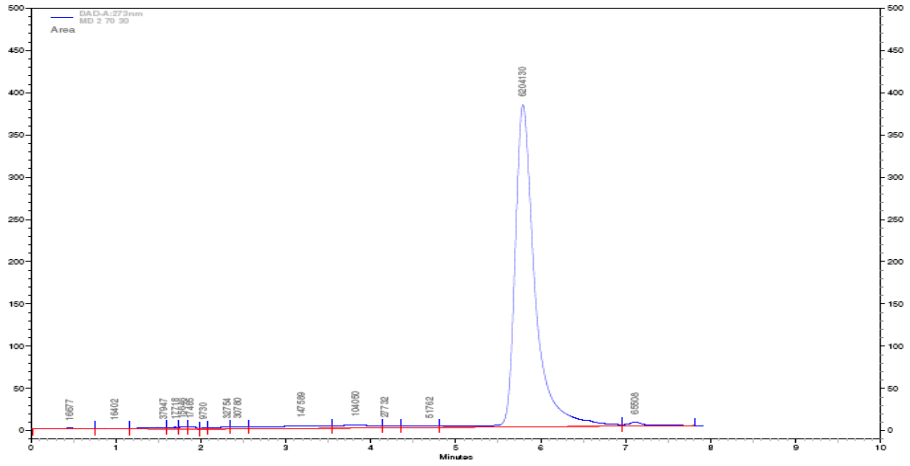
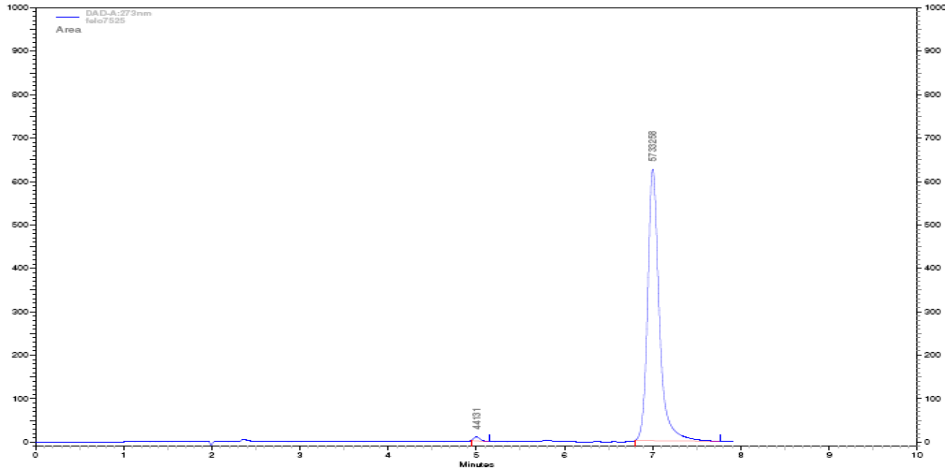



**Fig.2 and fig-3 Method development chromatogram Of (Acetonitrile:Water) 60:40&70:30**



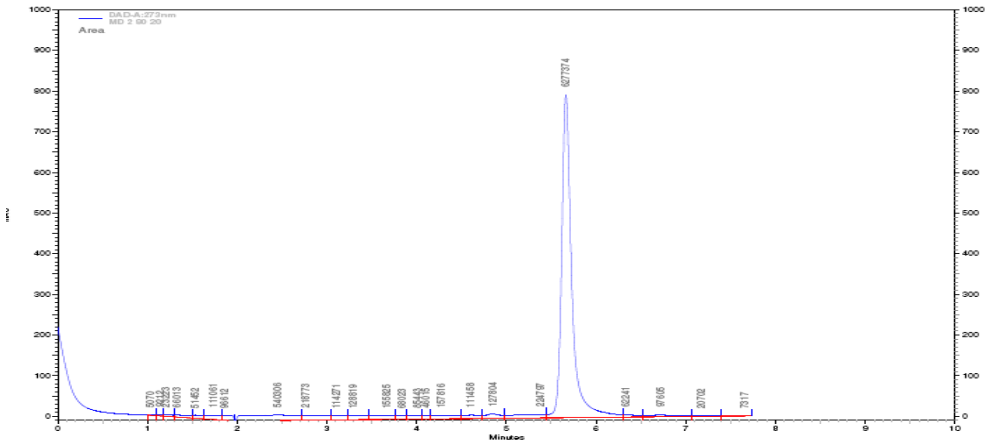




**Fig.4 and Fig-5 Method development chromatogram of 75:25&80:20 (Acetonitrile: Water)**

## Method Validation Parameters

### *3.1. Linearity:*

Method was shown perfectly in the range of 100µg/ml to 6.25 µg/ml. $R^2$ value found to be 0.9971 indicating perfect linearity. Unknown concentration of a drug was calculated using the following straight line equation obtained from the linearity curve.

**Table:2**

| **CONC.µg/ml** | 100 | 50 | 25 | 12.5 | 6.25 |
|---|---|---|---|---|---|
| **Trial-1 (Peak area)** | 508123 | 247059 | 104746 | 39007 | 32576 |
| **Trial-2 (Peak area)** | 510511 | 245121 | 102489 | 43359 | 33515 |
| **Average** | 509317 | 246540 | 103617 | 41183 | 33045 |

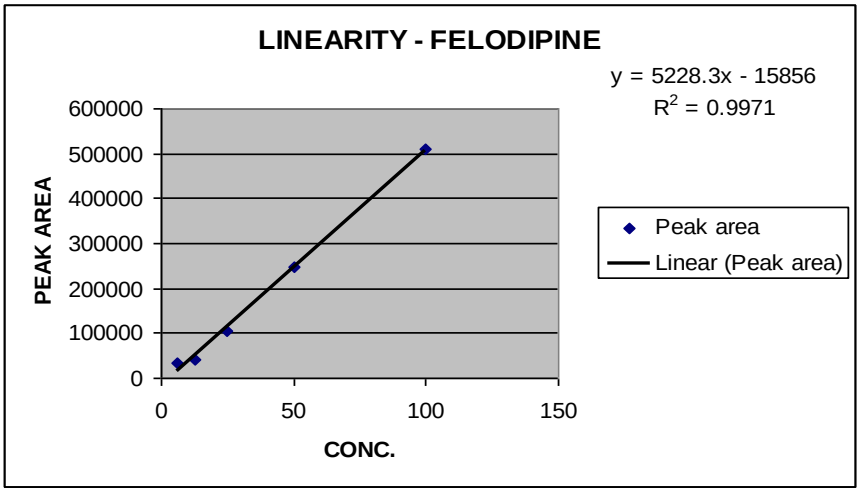


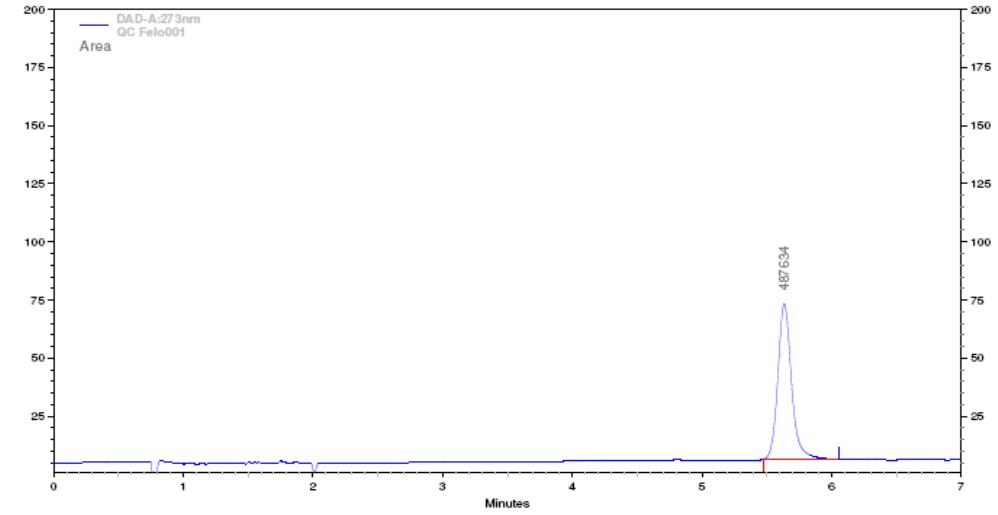

**Fig-6 Linearity Graph of Felodipine**

### *3.2. Accuracy:*

QC samples are taken in the concentration of 90, 70 and 40µg/ml, intermediate to standard concentrations, and area was calculated using the standard graph. Percentage deviation from the theoretical concentration and area was calculated.

**Table-3**

| **Injection Conc.(µg/ml)** | **Peak Area** | **Concentration** | **Percentage** |
|---|---|---|---|
| 90 | 491977 | 91.06 | 98.83 |
| 70 | 416659 | 76.66 | 91.31 |
| 40 | 226664 | 40.32 | 99.20 |



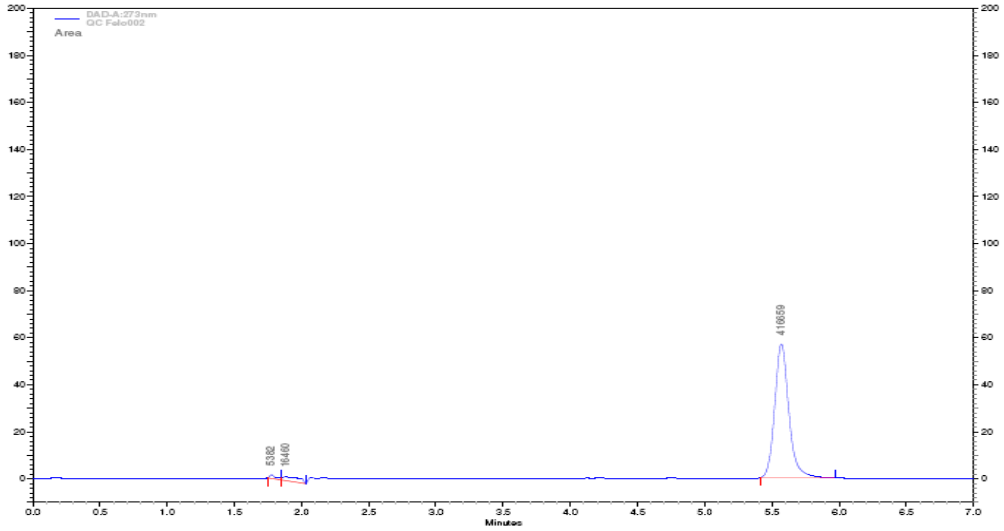




**Fig-7 Accuracy chromatogram of 90 µg/ml Fig-8 Accuracy chromatogram of 70µg/ml**

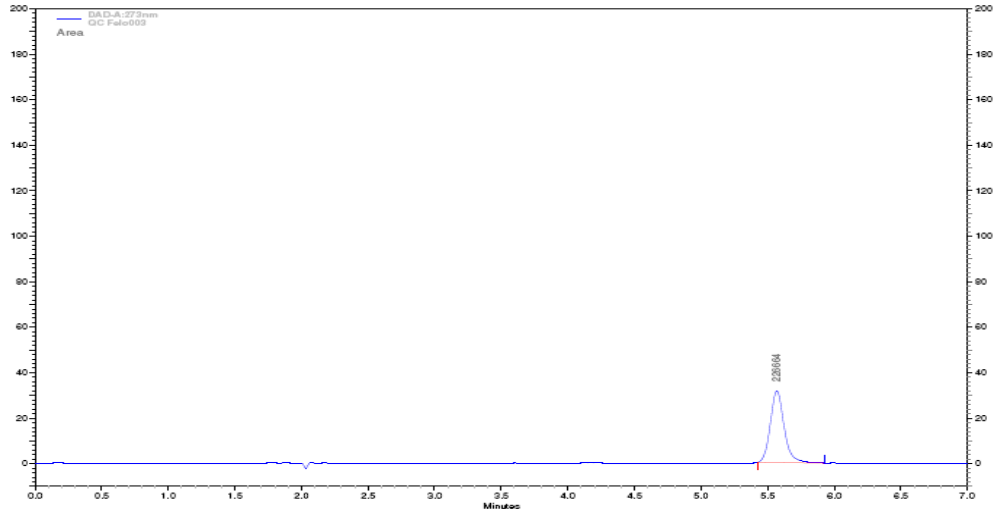



**Fig-9 Accuracy chromatogram of 40µg/ml**

***3.3.Injection Precision :***

RSD value for six injection of the same concentration was found to be 50µg/ml indicating precision of the injector as well as the method.

**Table-4**

| **Injection** | **Peak Area** |
|---|---|
| Injection-1 | 278663 |
| Injection-2 | 280153 |
| Injection-3 | 282377 |
| Injection-4 | 283138 |
| Injection-5 | 287083 |
| Injection-6 | 290677 |
| **Mean**<br>**SDV**<br>**RSD** | 243795.1<br>4475.265106<br><br>1.835665944 |

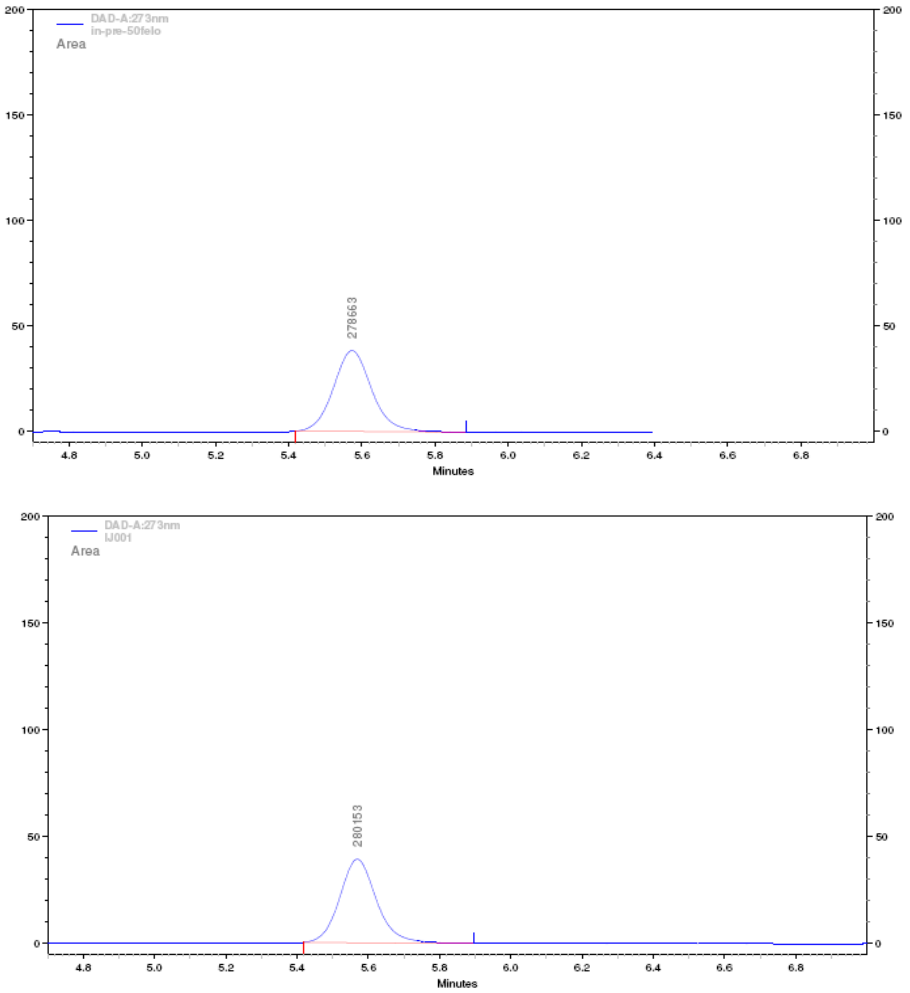
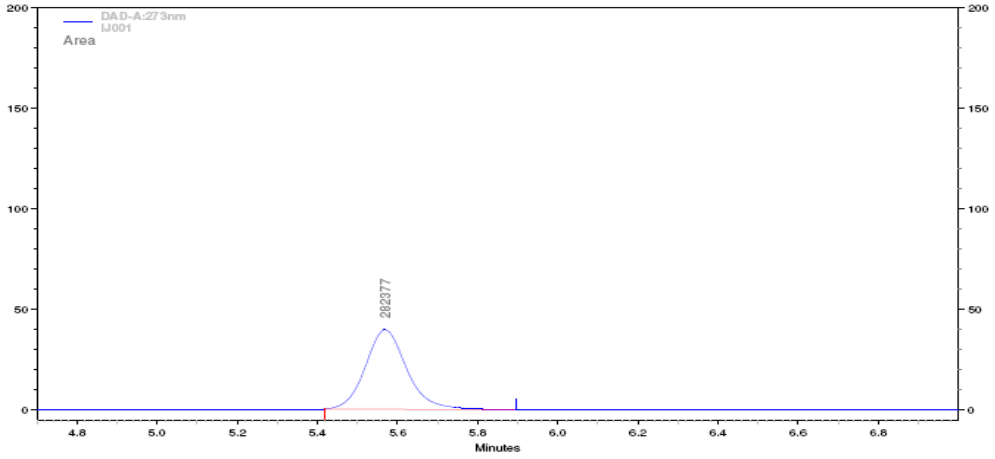



**Fig- 10 Injection Precision chromatogram of 50µg/ml (Trial-1) Fig- 1150µg/ml (Trial-2)**



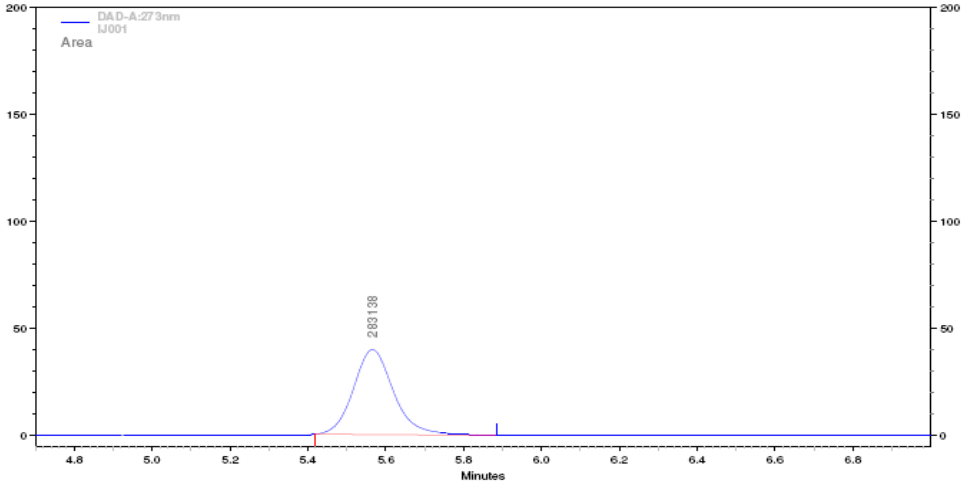
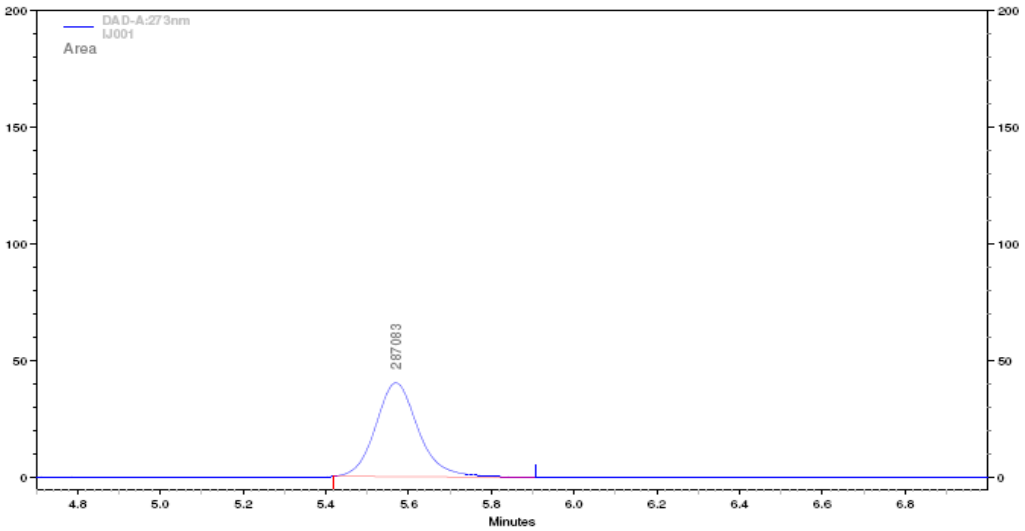




**Fig- 12 Injection Precision chromatogram of 50µg/ml (Trial-3) Fig- 13 50µg/ml (Trial-4)**



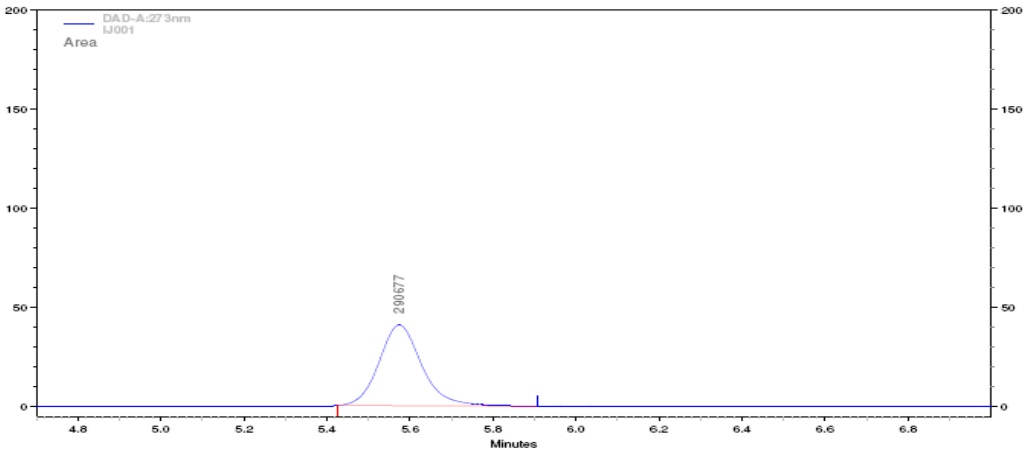



**Fig- 14Injection Precision chromatogram of 50µg/ml (Trial-5) Fig- 14 50µg/ml (Trial-6)**

### *3.4.Intraday and Interday:*

To evaluate intraday and Interday variation 200µl of Felodipine was injected in duplicate in two consecutive days and area was calculated . To evaluate accuracy both sample were subjected to T-test .

**Table- 5**

| **Concentration** | **100 µg/ml** | **25 µg/ml** |
|---|---|---|
| Trial – 1&2 (peak area)<br>Date- 22-01-2-2010 | 508123<br>510511 | 104746<br>102489 |
| Trial – 1&2(peak area)<br>Date- 25-01-2010 | 596336<br><br>598994 | 130599<br>130100 |
| **T- Test** | 0.000204341 | 0.00093201 |

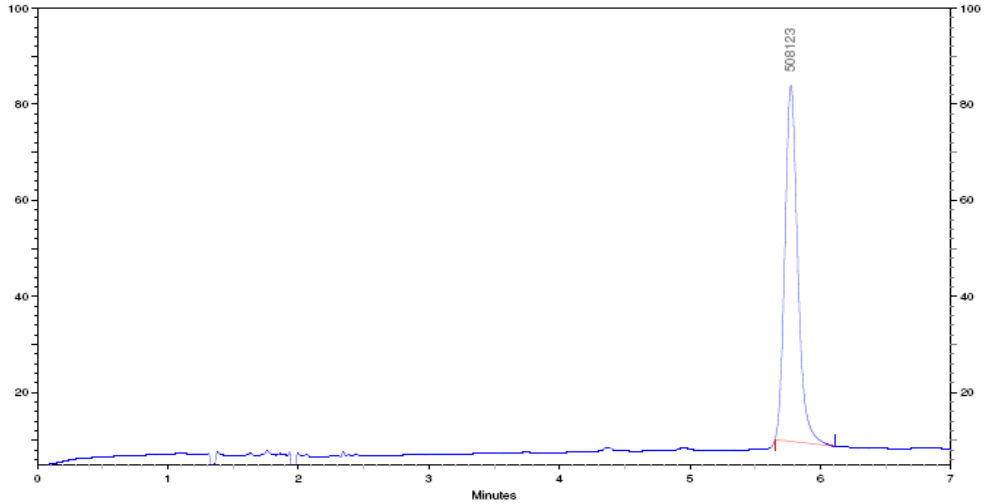



**Fig-15 Intraday Chromatogram of 100 µg/ml Trial-1**

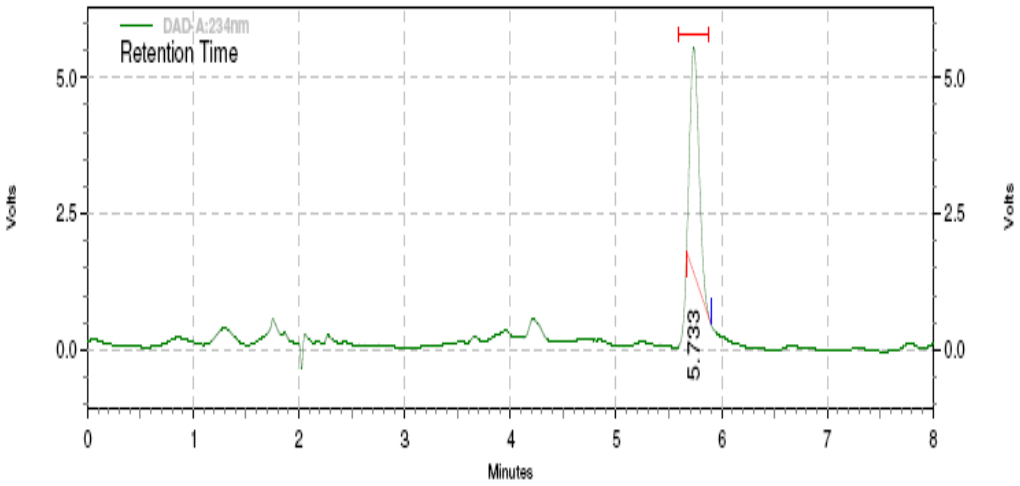
### *3.5.LLOD &LLOQ:*

LLOD and LLOQ were found to be 3.125ug and 1.562ug respectively where Signal/Noise was found to be 0.922 , 3.076 and0.194,0.647 respectively.

**Table-6**

| Concentrations(ug) | LLOD | LLOQ |
|---|---|---|
| 3.125 | 0.922 | 3.076 |
| 1.562 | 0.194 | 0.647 |

**Table showing concentrations of LLOD and LLOQ**



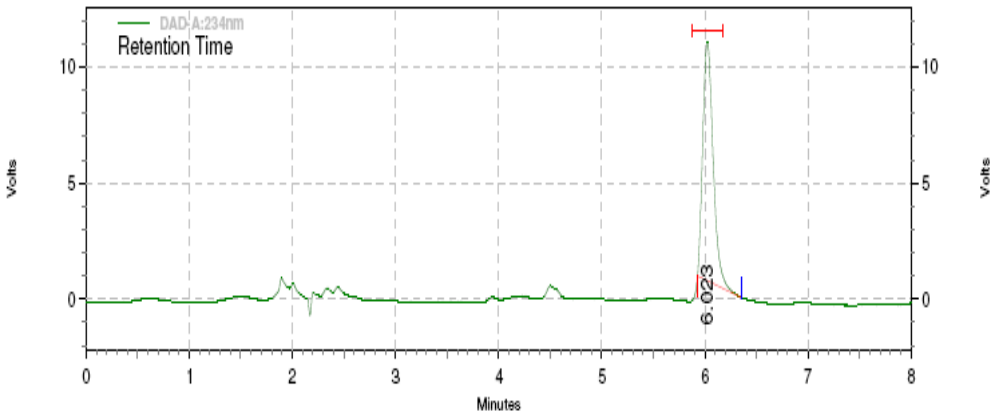




**Fig-16 chromatogram of LLOD** **Fig.17 showing chromatograms of LLOQ**

**Table-8**

| SL.no | Injected conc.(ug) | Recovery conc. | Recovery % |
|---|---|---|---|
| 1 | 25 | 26.13 | 96.1 |
| 2 | 12.5 | 12.80 | 97.6 |

**Table showing Quality of a drug Felodipine**

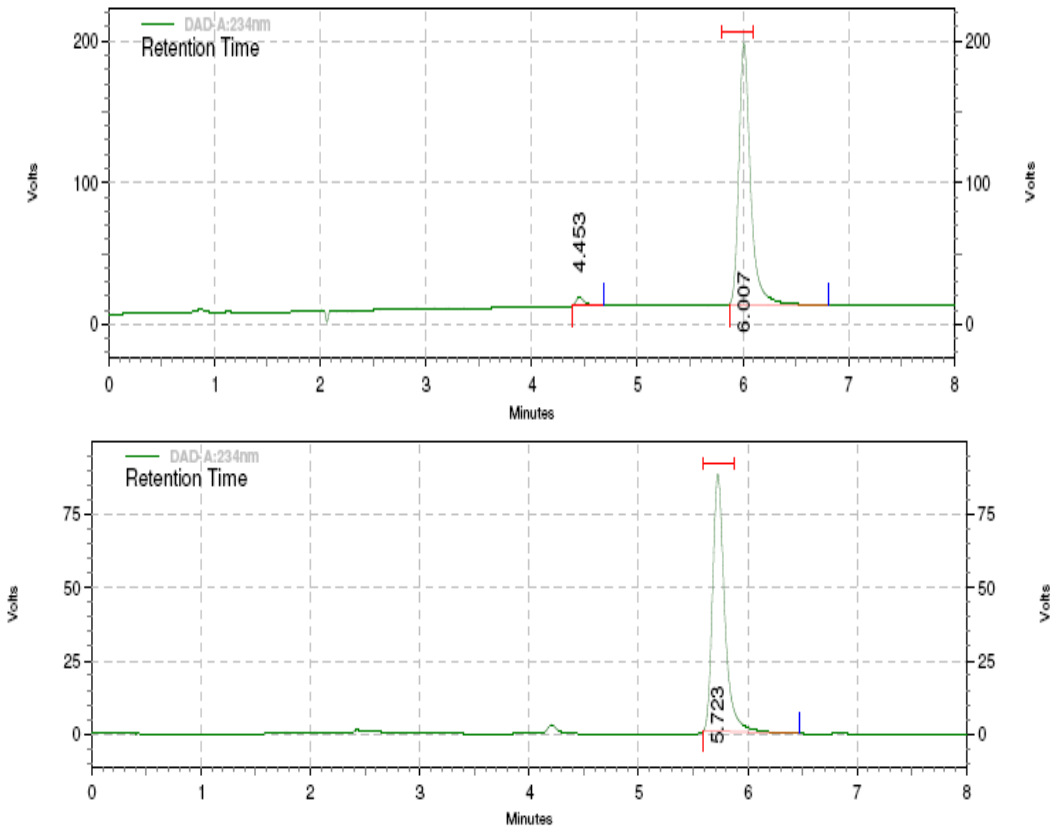



**Fig.18 peak areas of drug felodipine(plendil)**

***4.1.Photo stability studies:***

After the establishment of optimized HPLC conditions and validation parameters for felodipine , stability studies were performed . Felodipine drug dissolved with the solvent of (Acetonitrile:water) and then subjected to HPLC and peak areas were recorded . The concentration till 5 hrs are presented in the below graph.

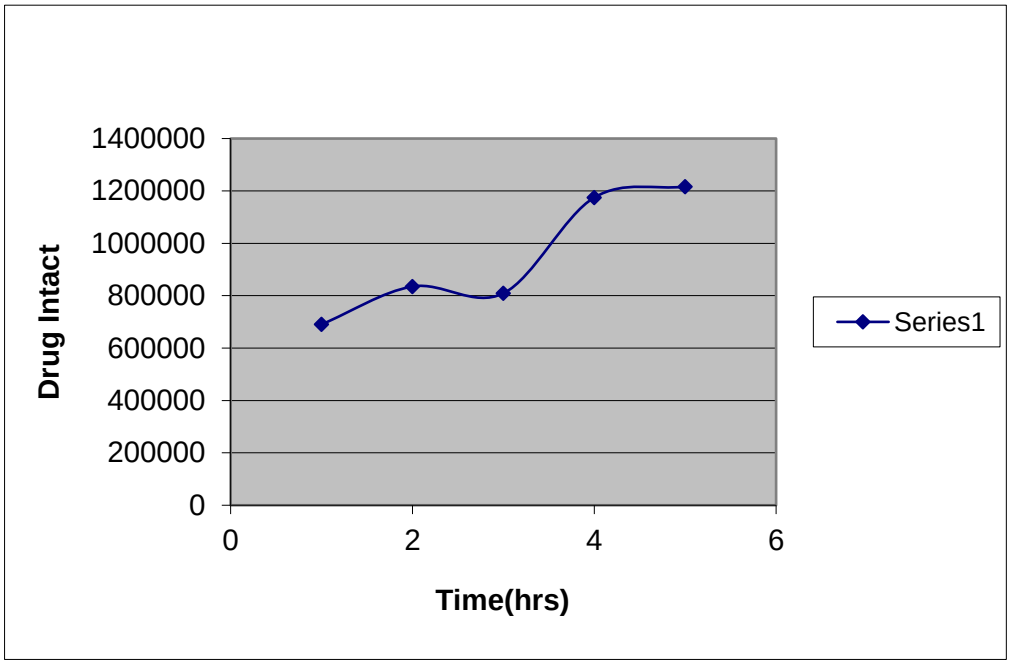
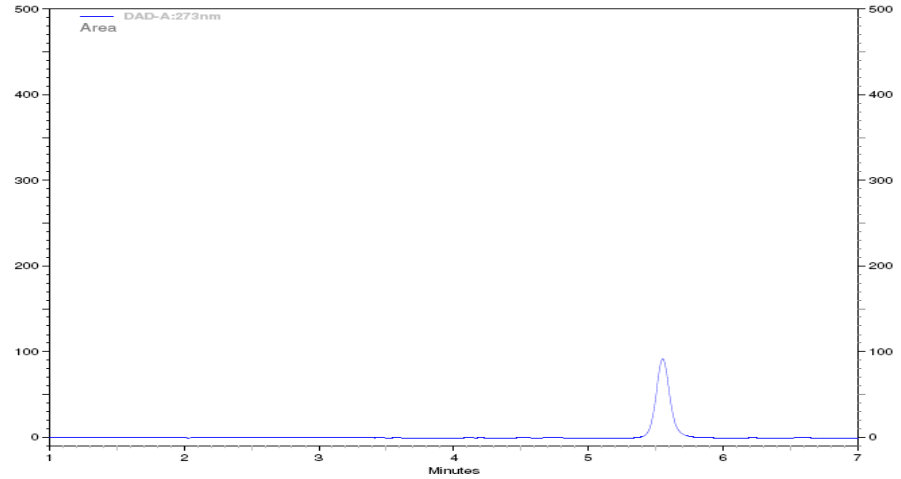



**Fig-19Photodegradation graph of felodipine**

From the figure it was cleared that intact drug concentration increased with increased time. Its cleared that there was no significance loss of drug concentration till up to 5 hrs . The concentration was increased because the evaporation of solvent due to sun light.



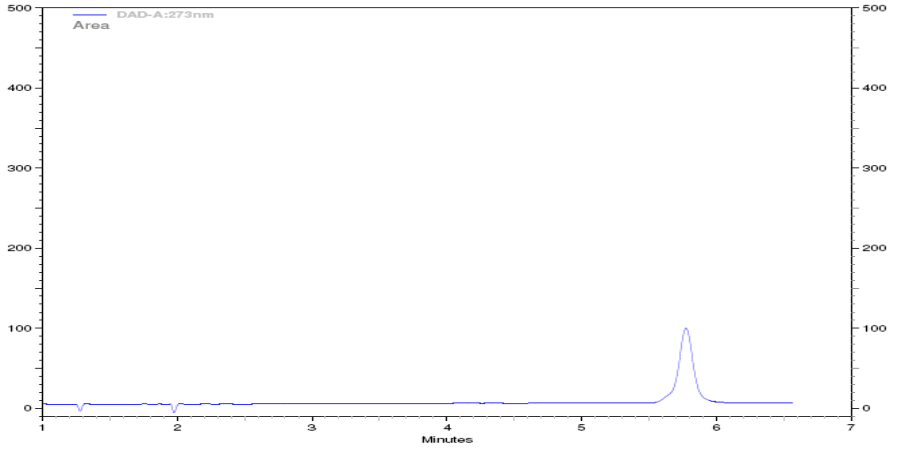
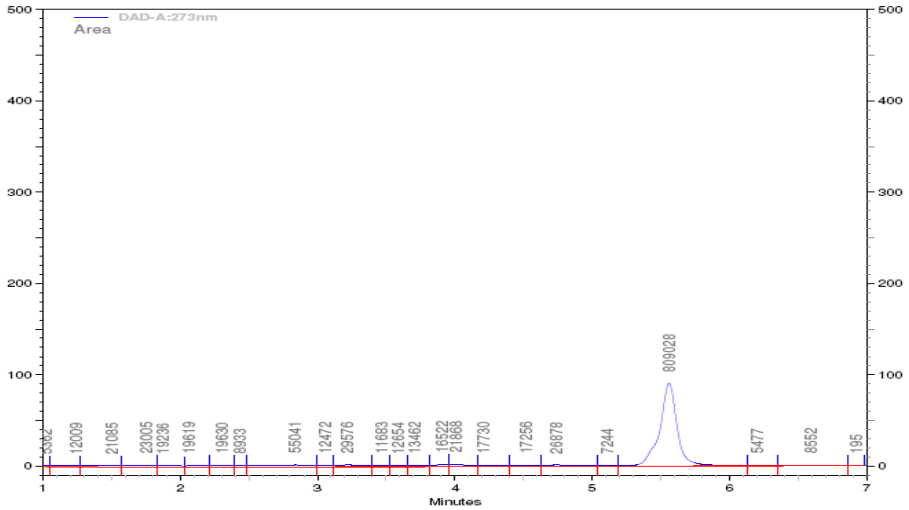




**Fig-19 Chromatogram of photo degradation after 1 hr** **Fig- 20 After 2 hrs**



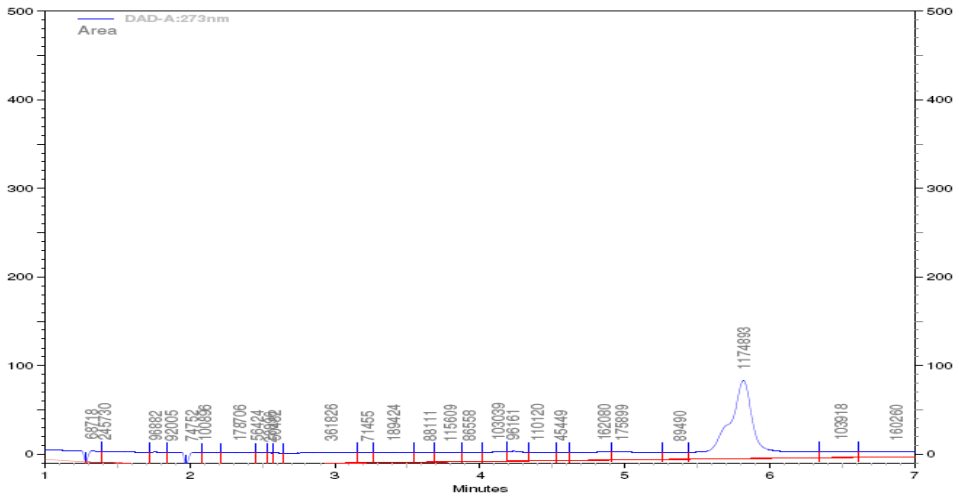



**Fig-21 Chromatogram of Photo degradation after 3 hrs Fig-22 after 4hrs**

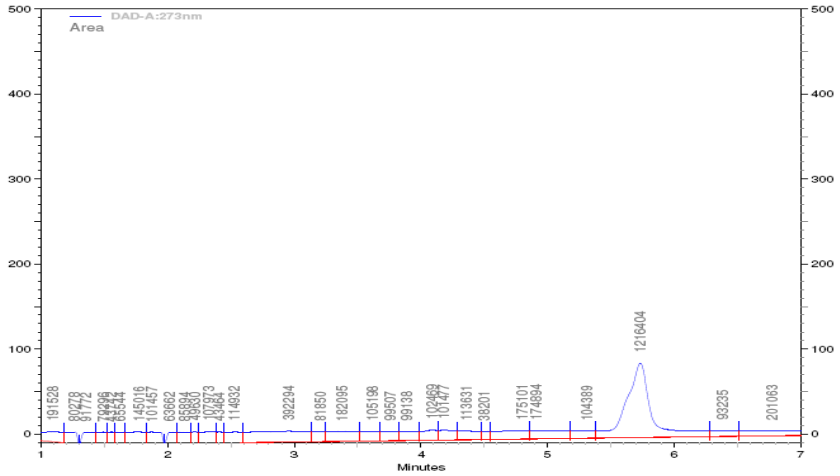



**Fig-23 Chromatogram of Photo degradation after 5hrs**

***4.2.Acid stability studies:***

The stability studies were performed , the drug felodipine was dissolved with 1N &2N HCL & Acetonitrile , then they are subjected to HPLC and peak area was recorded.

The percentage of the intact drug concentration of Dissolved with 1N HCL for up to 40 min.

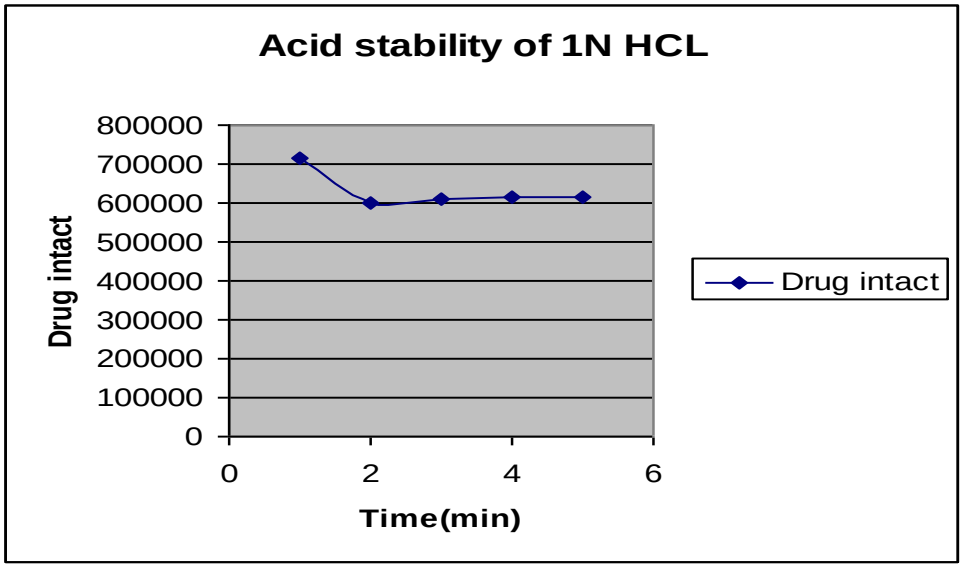


**Fig-24Acid degradation graph of 1N HCL**

From that figure it was cleared that intact drug concentration decreased with increased time . Its interesting to record that there was significance loss in intact drug concentration at 30 min. Nearly 18% of the intact drug concentration was lost and extra peak was observed in chromatograms.

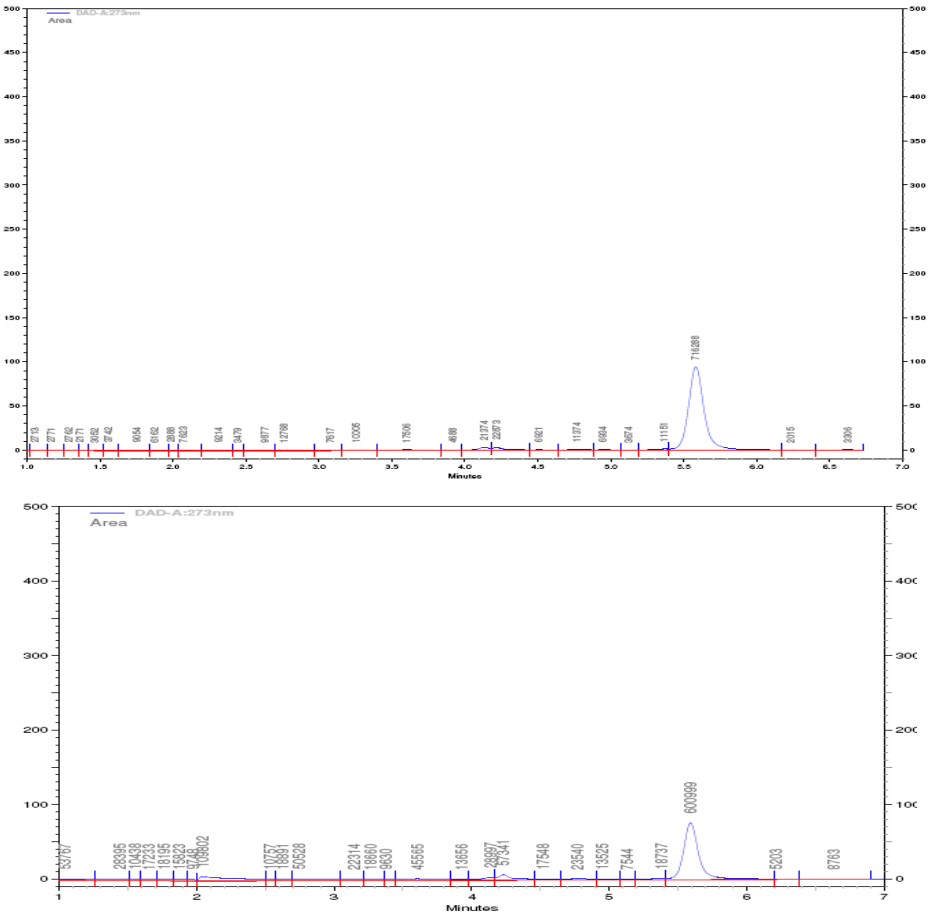
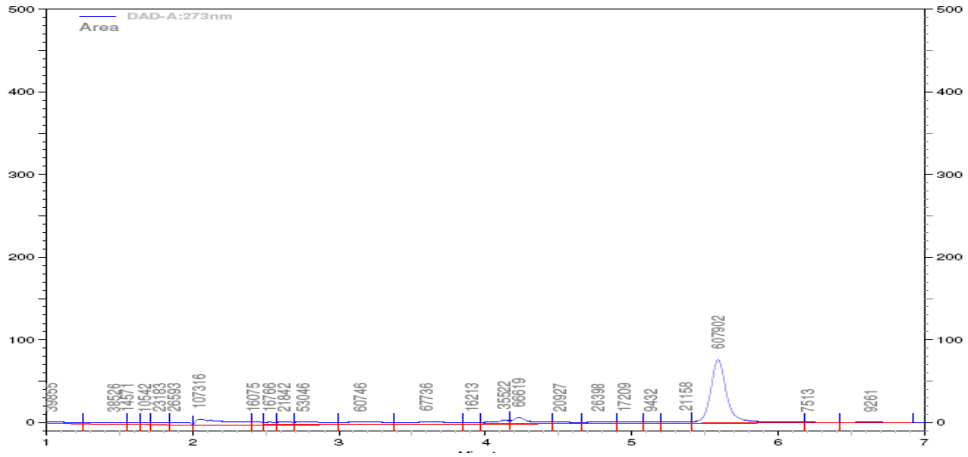



**Fig- 25 Chromatogram of Acid degradation with 1N HCL( 0 min) Fig-26 1N HCL (10 min)**



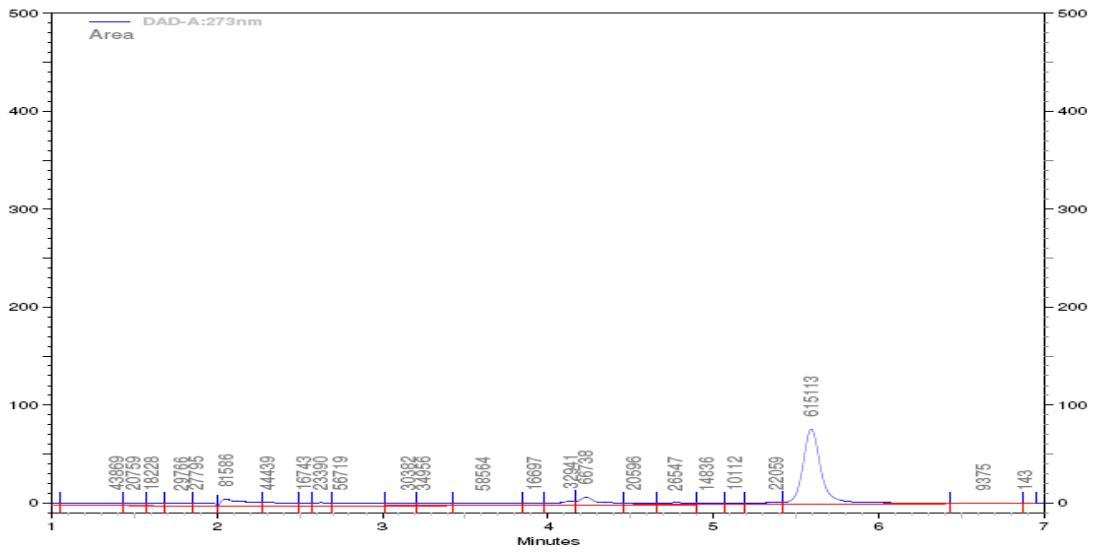



**Fig-27&28 Chromatogram of Acid degradation with 1N HCL (20min) & with 1N HCL (30 min)**

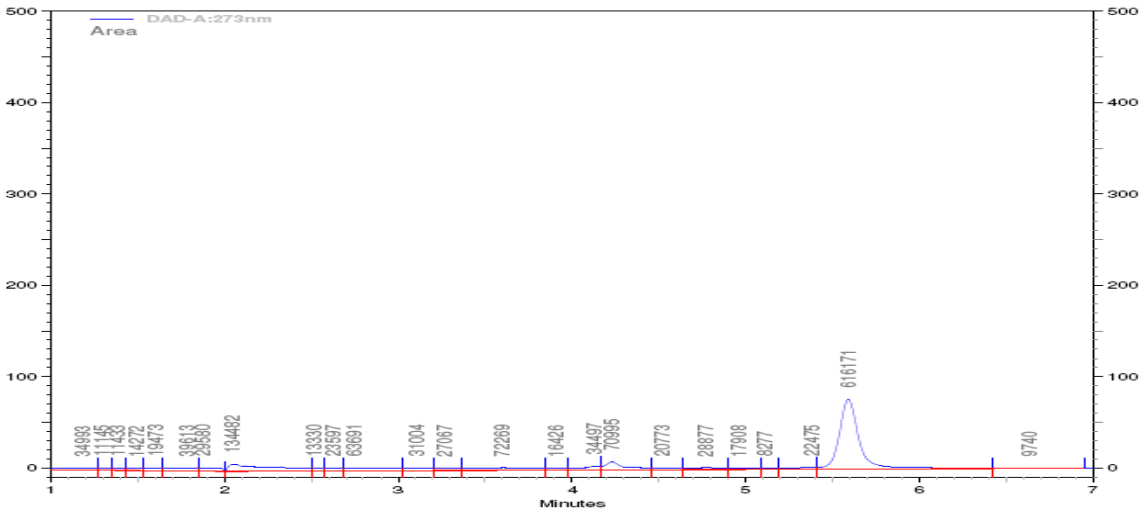



**Fig-29 Chromatogram of Acid degradation 1N HCL (40 min)**

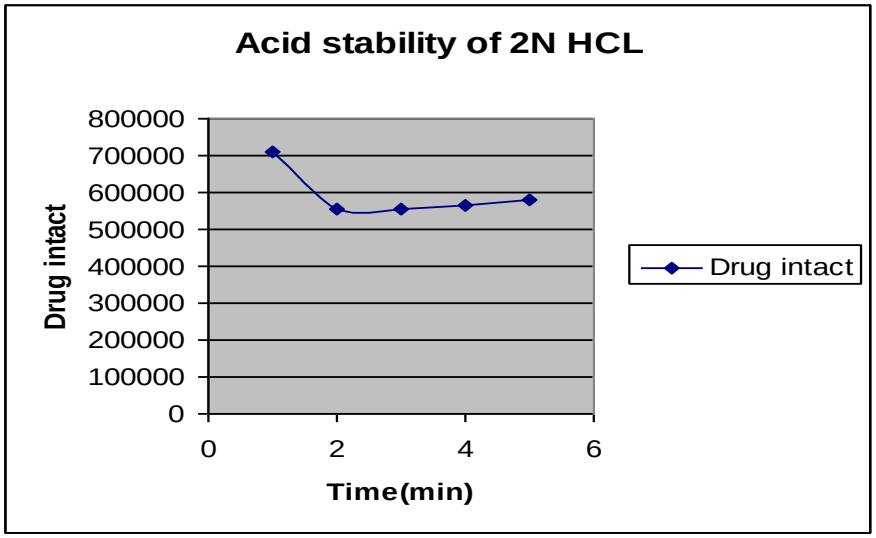


**Fig-30 acid stability of 2N HCl**

From this graph it was cleared that , the felodipine was dissolved with 2N HCL & Acetonitrile, then subjected on HPLC , the result is decreased the 40% drug concentration after increased the time at 10 min .

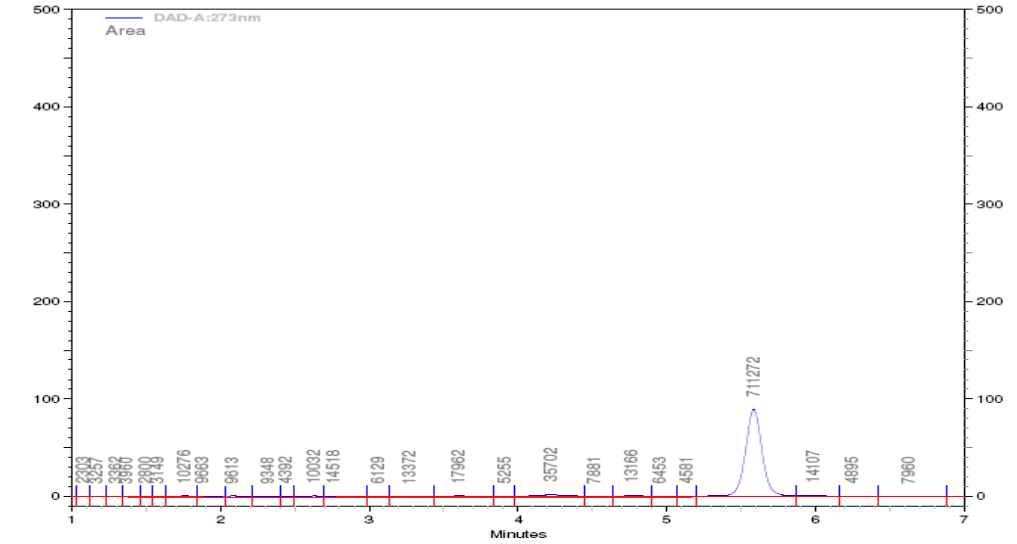
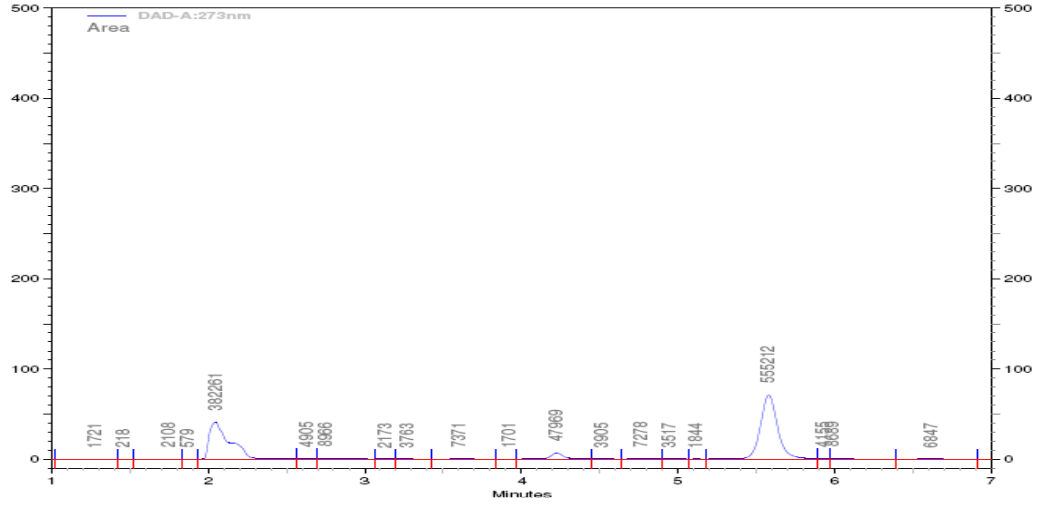



**Fig-31 Chromatogram of Acid degradation 2N HCL (0min) Fig- 32 2N HCL (10min)**



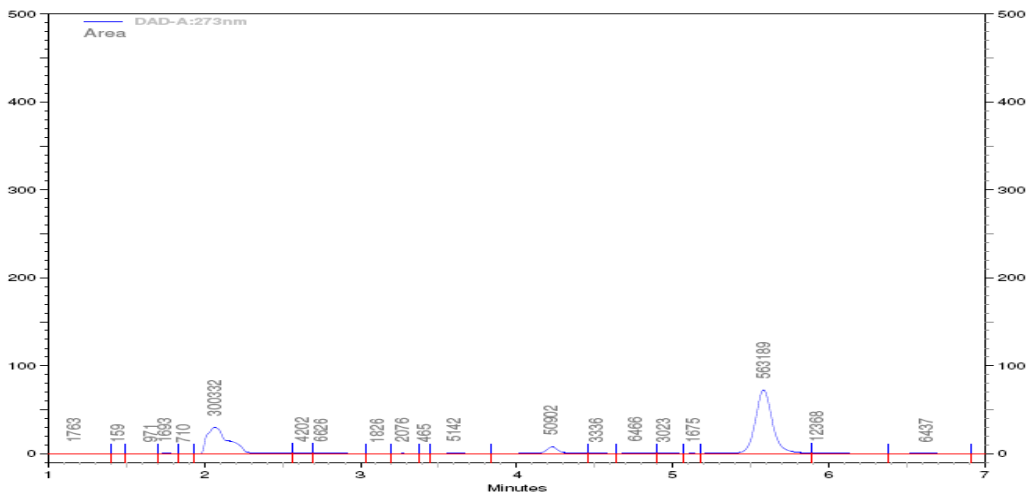



**Fig-33 Chromatogram of Acid degradation 2N HCL (20min) Fig-34 2NHCL (30 min)**

***4.3.Thermal stability studies:***

The thermal degradation studies was occurs , Felodipine was dissolved with the solvent of (Acetonitrile:Water) , then placed under dry bath at 40 & 55°C of different time interval up to 2 hrs respectively.

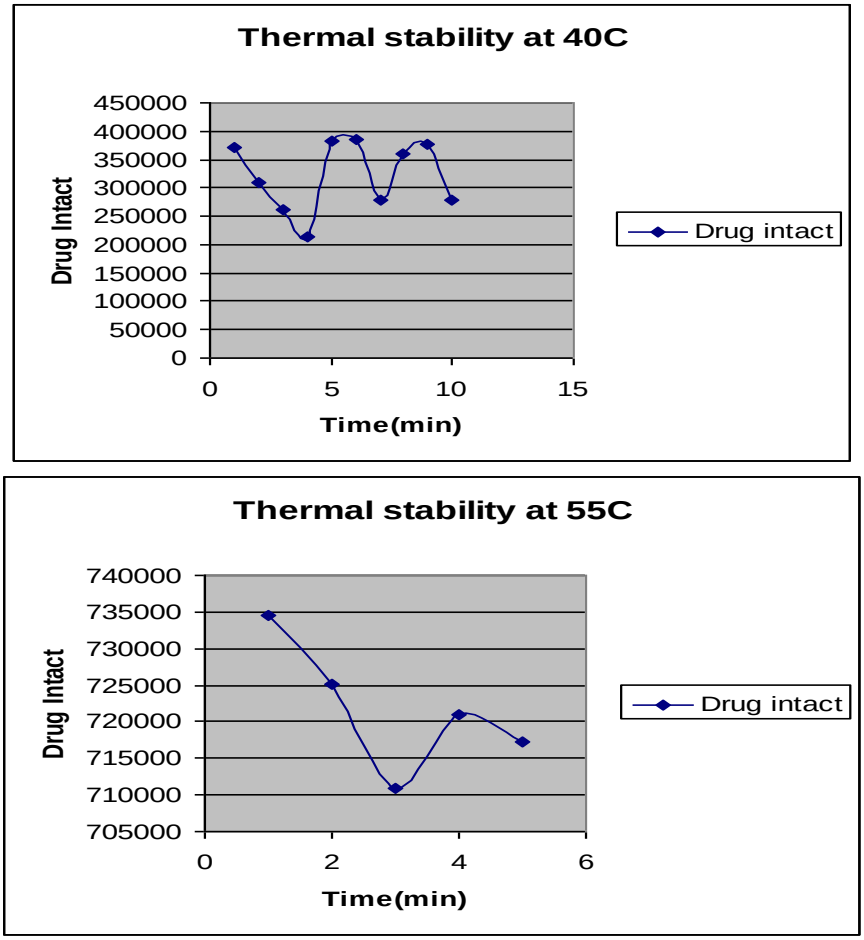



**Fig-34 and 35 thermal stability at 40 and 55$^0$C**

## 5. Conclusion :

The study was generates large amount of quality data which serves as highly powerful and convenient analytical tool.All the parameters were validated as per ICH guidelines and found to be within the acceptance criteria . So the developed method may be recommended for routine analysis in research institutions and quality control departments in industries for the determination of felodipine its oral formulations.

After the establishment of optimized HPLC condition and variation parameters for felodipine, stability studies were performed. In the first phase drug was exposed to direct sun light up to 5 hrs and subjected to HPLC, the result is no degradation occurred. Further stability of drug was evaluated under acidic condition. From our result it was cleared that felodipine was degraded under (2 N HCL: Acetonitrile) after 5 min. Also stability of drug was evaluated under different thermal condition. From our result it was cleared that felodipine was stable at 55°C up to 2hrs.

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